Developing nanofibrous sheets to scavenge storage lesion to enhance the quality and shelf-life of stored Red Blood Cells and prevent transfusion-related complications

Synopsis of the thesis submitted for the partial fulfilment of the degree of Doctor of Philosophy

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Synopsis

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Blood transfusion is a pivotal practice performed frequently in critically ill patients with anaemia (7-8g/dl hemoglobin)[1]. According to the data published by the National Aids Control Organization (NACO) in 2017, 6.57 lakh liters of blood (roughly 53 water tankers) were discarded in the last five years. One of the main reasons that can be cited for dumping this enormous volume of collected blood was the deterioration of blood quality during the storage time of 35-42 day[2]**.** Previous investigations in cardiac research have reported an increase in severe complications and mortality when RBCs stored for > 2 weeks were used for transfusion[3]. These increased complications are attributed to the accumulation of a wide range of storage lesions, also known as Damage Associated Molecular Patterns (DAMPs) in stored blood over time, limiting the shelf life of stored RBCs. Transfusion of old stored whole blood consists of a pool of stored lesions that are primary mediators in the pathophysiology of fatal diseases like Transfusion-Related Acute Lung Injury (TRALI), deep vein thrombosis, and organ dysfunction[4,5]**.** It has been long established that leucocytes associated with DAMPs in donor blood are potential immune elicitors in recipients. To reduce the accumulation of leucocytes-associated DAMPs like NETs, RBC units are treated with leukoreduction filters prior to storage [6]. However, this technology does not profess to eliminate all DAMPs, especially those contributed by erythrocytes. Past investigations suggest that adding preservatives, storing RBCs in an alkaline hypotonic solution with antioxidants, or rendering anaerobic storage conditions improves storage time up to 10-15 days[7–10]**.** Still, these efforts do not exclusively focus on removing RBC and WBCs associated lesions and improving blood quality or shelf life. Since no practical approach is listed in the field that claims to remove all classes of storage lesions, there is a need to develop a technology to solve this unmet clinical need. ***Therefore, the aim of the work is to improve the quality of NLR (Non-leukoreduced) & LR (Leuko reduced) stored RBCs and increase their shelf life by scavenging the wide range of DAMPs by using a biomaterial platform. This may also efficiently reduce transfusion-related complications such as TRALI.***

This thesis contains four chapters, and below we have described the contents of each chapter.

Chapter 1: Introduction - An overivew of existing strategies and technologies to enhance the quality of stored blood

In Chapter 1, we reviewed all the strategies and technologies that were developed to enhance the quality of stored blood. We have comprehensively reviewed all technologies developed in the field.

Furthermore, existing state-of-art technologies, and the potential limitations of those technologies have been discussed in detail.

Chapter 2: Intermittent scavenging of storage lesions from stored red blood cells by electrospun nanofibrous sheets enhances the quality and shelf-life of stored RBCs

Transfusion of healthy RBCs is a lifesaving process. However, upon storing RBCs, a wide range of DAMPs, such as cell-free DNA, nucleosomes, free-hemoglobin, and poly-unsaturated-fatty-acids (PUFAs) are generated. DAMPs can further damage RBCs. Thus, the quality of stored RBCs declines during storage and limits their shelf-life. These DAMPs contain either positive or negatively charged species (**Figure 1a**). Hence, we developed taurine and acridine containing electrospun-nanofibrous sheets (*Tau-Acr*NFS), consisting of anionic and cationic charges on their surfaces.

Here, we show in **Figure 1b** that *Tau-Acr*NFS are efficient in scavenging DAMPs from stored human and mice RBCs, *ex vivo*. Furthermore, intermittent scavenging of DAMPs by *Tau-Acr*NFS during the storage, prevented the loss of RBC membrane integrity and reduced discocytes-*to*-spheroechinocytes transformation in stored-old-RBCs. Additionally, RBC-transfusion studies in mice reveal that intermittent removal of DAMPs enhanced the quality of stored-old-RBCs equivalent to freshly collected RBCs and increased their shelf-life by ~22%.

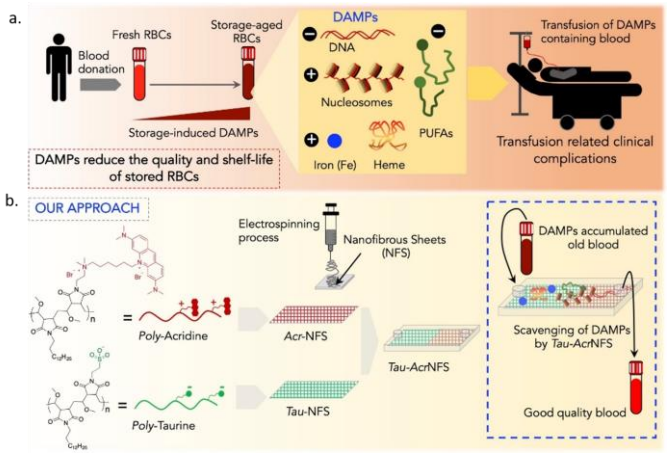

Figure1: a) Schematic representation of the presence of DAMPs progressively reduces the quality of stored RBCs and limits their shelf-life. All DAMPs contain either positive or negatively charged entities. **b)** Using polymers, the charge-bearing electrospun nanofibrous sheets (*Tau*-NFS and *Acr*-NFS) were prepared to scavenge charged DAMPs from stored RBCs, which remarkably enhanced the quality and shelf-life of stored RBCs.

In summary, our study implicates in situ generated DAMPs in causing accelerated deterioration of RBCs. Furthermore, we established a novel approach to slow down the damage of stored RBCs through intermittent scavenging of the storage lesion.

<u>Chapter 3</u>: Improving the quality of leukoreduced RBCs by scavenging erythrocytes-associated storage lesions by electrospun nanofibrous sheets

Our study was not restricted to the NLR(Non-leukoreduced) RBCs unit. The kinetic study of DAMPs production in Leukoreduced (LR) units was also investigated. Although the Leucocytes-associated DAMPs production rate was not significant as NLR units, the release of free hemoglobin and bioactive lipids raised our concern.

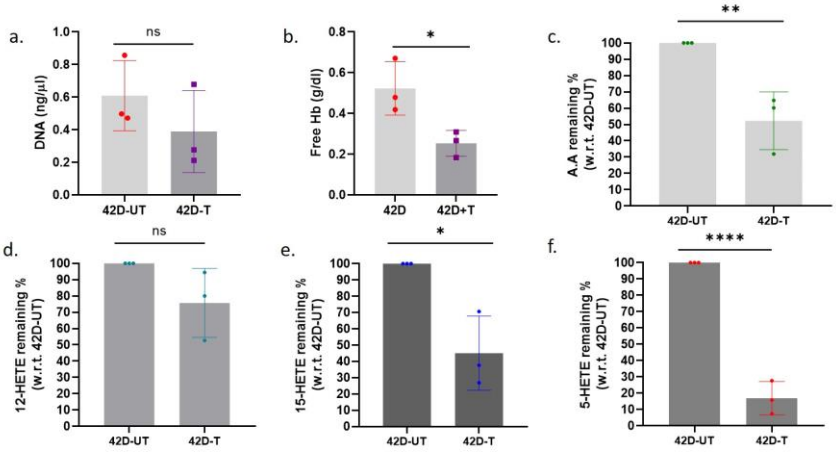

Figure 2: *Tau-AcrNFS significantly eliminates RBCs associated DAMPs:* **a)** There was no significant change in the concentration of free DNA in 42nd day LR unit post treatment since the production of free DNA was relatively low in 42nd Day LR units. **b-f)** A remarkable drop of Free Hemoglobin (Hb), Arachidonic acid (AA), 5 &15-HETE value after scaffolds treatment provides a method to purify LR blood on 42nd day that contains soluble bioactive lipids generated from RBCs.

Similar to intermittent treatment of NLR blood, LR blood was also intermittently treated (21st /28th day) and DAMPs production were quantified on 42nd day. The production of extracellular DNA in LR blood units was low, as expected by the end of 42nd Day due to the removal of leukocytes (**Figure 2a**). A critical trend has been witnessed in free hemoglobin (Hb) production, which is increased up to 10 folds giving an idea that leukofilters had no control over the accumulation of RBC-associated DAMPs. Thus, cleaning LR units with *Tau-Acr*NFS before transfusion could significantly reduce the formation of RBCs associated lesions. The data suggest that free Hb was significantly reduced to 50% post-treatment (**Figure 2b**). Additionally, significant production of AA and its metabolites was observed, however, scaffold treatment reduced its concentration up to 40-50% (**Figure 2c-f**). Herein, the concept of intermittent treatment worked quite efficiently where the free Hb and bioactive lipids concentration was significantly reduced when treated on the 21st/ 28th day of storage.

In summary, a remarkable drop of erythrocytes associated DAMPs after scaffolds treatment provides a novel method to purify and maintain the quality of LR blood even after leukoreduction.

<u>Chapter 4:</u> Selective alleviation of DAMPs from stored blood products using nanofibre scaffolds to rescue transfusion related acute lung injury (TRALI)

Transfusion Related Acute Lung Injury (TRALI) is a rare complication of blood transfusion that occurs during or within 6 hours of transfusion of stored blood component[11]. Herein, we developed a "Two-Hit non-antibody-mediated TRALI model" using 17th Day stored mice blood after the first hit of LPS (**Figure 3**). The rationale for storing mice blood up to 17days is extrapolated from our previous study, where we have shown that the twice periodic cleaning of DAMPs with *Tau-Acr*NFS can enhance the shelf life of stored mice RBCs from 14 Days to 17 Days. So, revisiting the same concept and aggravating the incidence of TRALI events, we have transfused 17th Day untreated (17D-UT), 17th Day old blood end day treatment (17D-ET), and intermittently treated 17th Day old blood (17D-IT) in LPS challenged mice.

Our data suggested that transfusing 17D(IT) blood does not stimulate the second event of TRALI.

The concept of removing DAMPs also explores that the stored blood needs to pass through scaffolds before transfusion (bedside purification) to reduce TRALI incidence.

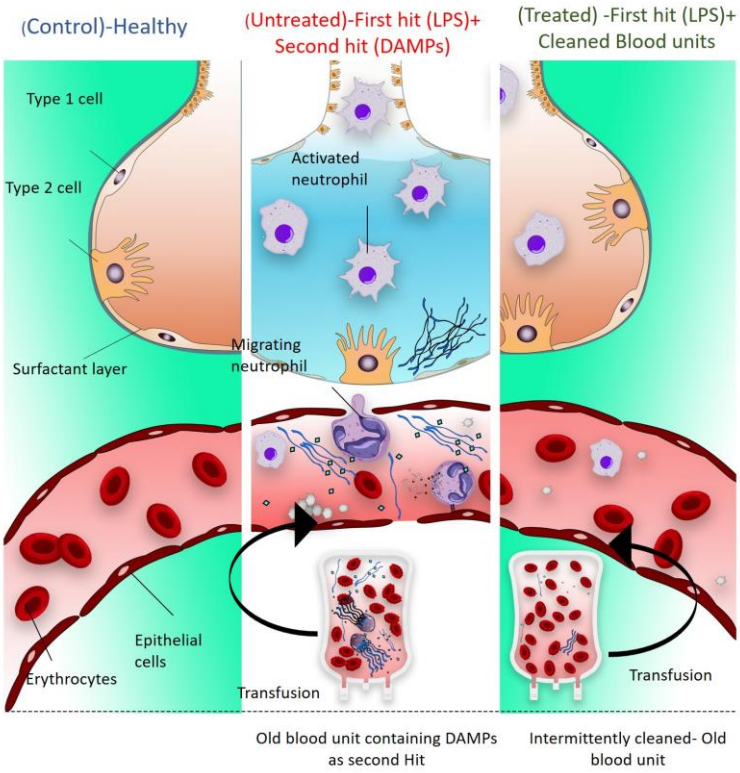

Figure 3: The control group shows the normal condition of lung and blood vessels, The untreated group comprises the two-hit non-antibody-mediated model of transfusion-related acute lung injury (TRALI) with hypothesized pathophysiology. The treated group describes the concept of treatment with *Tau-Acr*NFS improves the quality of 17D old mice blood and when transfused in LPS challenged mice did not lead to TRALI.

In summary, the model's and treatment validity are confirmed based on evaluating specific clinically relevant endpoints. The periodic removal of DAMPs from old blood and their transfusion shows no histological evidence of lung injury, endothelial barrier permeability, and inflammation in mice compared to untreated TRALI mice.

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Student's Publications:

1. **Pandey S.**, Mahato M., Srinath P., Bhutani U., Goap T., Ravipati P., & Vemula P. K*. (2022) Intermittent scavenging storage lesion from stored red blood cells by nanofibrous sheets enhances the quality and shelf-life of stored RBCs. ***Nature Communications*** 2022 (Under Review, **NCOMMS-22-24335)**
2. Thorat K, **Pandey S**, Chandrashekharappa S, Vavilthota N, Hiwale AA, Shah P, Sreekumar S, Upadhyay S, Phuntsok T, Mahato M, Mudnakudu-Nagaraju KK, Sunnapu O, Vemula PK.* "Prevention of pesticide-induced neuronal dysfunction and mortality with nucleophilic poly-Oxime topical gel" ***Science Advances.*** 2018, *4*, eaau1780.
3. **Pandey S**., Kumar J*., Srivastava N. K., & Dutt S. Homology Modeling of DNA polymerases of Herpesviridae family and structure-based virtual screening for inhibitor identification. ***Journal of Applied Pharmaceutical Science*** 2015**,** *5*(12), 048-055.

Patents:

1. Vemula PK, Mahato M, **Pandey S**, Srinath P, Bhutani U. "Compositions and methods to enhance the quality and shelf-life of biological materials" **Indian Provisional Application: 202241014827 (Filed Date: 17-03-2022)**
2. Vemula PK, Thorat K, Chandrashekharappa S, **Pandey S**. "Compositions, materials, and methods for deactivating toxic agents" International PCT Application**: PCT/IB2019/052296 (Granted:26-11-2021)**
3. Vemula PK, Thorat K, Chandrashekharappa S, **Pandey S**. "Compositions, materials, and methods for deactivating toxic agents" **Sri Lankan Convectional Patent Application Number: 20419 (Filed date: 21-03-2019)**

Awards:

1. Winner of **Instem Best poster award (2022) and international travelling grant** for presenting "Development of Nanofibrous scaffold to improve the quality and shelf life of stored RBCs
2. Won **3minute-Thesis Competition in Life Science Category at "SARANSH" (2021)**, a National Level Sciences communication organized by Indian Young Academy of Science (INYAS).
3. Won **Gandhian Young Technological Innovation Award-2019** for developing anti-pesticide topical gel technology.
4. Winner **of Instem Best poster award (2018) and international travelling grant** for presenting "Investigating the structure-activity relationship of amphiphilic nucleophiles to hydrolyze major classes of pesticides in micellar medium".